PRELIMINARY EXPERIMENTAL REARING OF MUSSEL (*MYTILUS EDULIS AOTEANUS, AULACOMYA MAORIANA* AND *PERNA CANALICULUS*) LARVAE IN THE LABORATORY

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ABSTRACT

Larvae of *Mytilus edulis aoteanus*, obtained from ripe mussels by induced spawning were reared for 90 days at 17°C to late veliger stage. The gastrula stage was reached after 8 h, trochophore after 20 h and straight hinge stage after 40 h when they measured 120 µm. Ripe *Aulacomya maoriana* spawned and the larvae were reared for 24 days at 17°C. The gastrula stage was reached after 13 h, trochophore after 33 h and straight hinge stage after 60 h when they measured 80 µm. *Perna canaliculus* failed to respond to spawning stimulation even after conditioning. Some spontaneous spawning did take place but the cultures were contaminated and did not survive past gastrula stage (12 h at 17°C). The facilities and the methods used are described, and possible reasons for the failure of the larvae to metamorphose and settle are discussed.

INTRODUCTION

Mytilid larvae were among the first bivalves reared successfully in the laboratory and Bayne (1976) reviewed the basic techniques used by a number of workers. Larvae from known parents reared under controlled conditions can be used to study the effects of numerous environmental factors upon their development and metamorphosis, as well as aid in positive identification of planktonic larvae. Such studies have increased in importance with the recent upsurge in commercial farming of mussels.

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In New Zealand there are two species of commercial mussels, *Mytilus edulis aoteanus* and *Perna canaliculus*. In Marlborough Sounds the abundance of planktonic larvae of both species are monitored, using Booth's (1977) tentative descriptions for identification, to forecast spatfall for the mussel farming industry (Jenkins 1979). There is however no published account of laboratory rearing of the larvae of either species in New Zealand.

The present attempts to rear mussel larvae formed part of a wider investigation into mussel biology and farming techniques (Tortell 1976a). It was the intention of the study to obtain larvae of *Mytilus edulis aoteanus* and *Perna canaliculus* in order to conduct experiments on settlement behaviour. Specimens of *Aulacomya maoriana* were fortuitously collected along with the two principal species.

Mytilus edulis aoteanus and *Aulacomya maoriana* spawned without any difficulty, however *Perna canaliculus* did not and gametes could not be obtained under controlled conditions even after a variety of stimuli were applied. Larvae of *Mytilus edulis aoteanus* and *Aulacomya maoriana* were reared to an advanced stage on a number of occasions but they failed to metamorphose and settle. This paper describes the methods used and the results obtained, and examines the possible reasons for the partial success.

METHODS

LARVAL REARING FACILITIES

The larval rearing unit at the Victoria University of Wellington, Marine Laboratory, Island Bay (Fig. 1) consisted of a conditioning rack, constant temperature baths, a supply of filtered and sterilized seawater, an axenic algal culture bank and a culture of mixed algae.

The holding and conditioning rack for adult mussels consisted of twelve clear perspex tanks of approximately 14 l capacity, each supplied with cold and heated seawater, and drained by an overflow on the side. Each tank could be disconnected from the drain and removed from the rack for cleaning. Cold water to the conditioning tanks came directly from the main reticulation of the laboratory (90,000 l total volume; 5,500 lh^{-1} flow) and was kept at 100-110 lh^{-1} per tank. Heated water was supplied by a system similar to that described by Walne (1964). The wooden heating tank was lined with fibreglass and coated with a polyester resin. Heating was achieved by three 3 kW Vitreosil immersion heaters. A flow of up to 50 lh^{-1} per tank could be maintained. Each tank received a supply of both cold and heated water and the final temperature in each tank was achieved by altering the proportions. Thus a range of water temperatures were available from the same source of heated water.

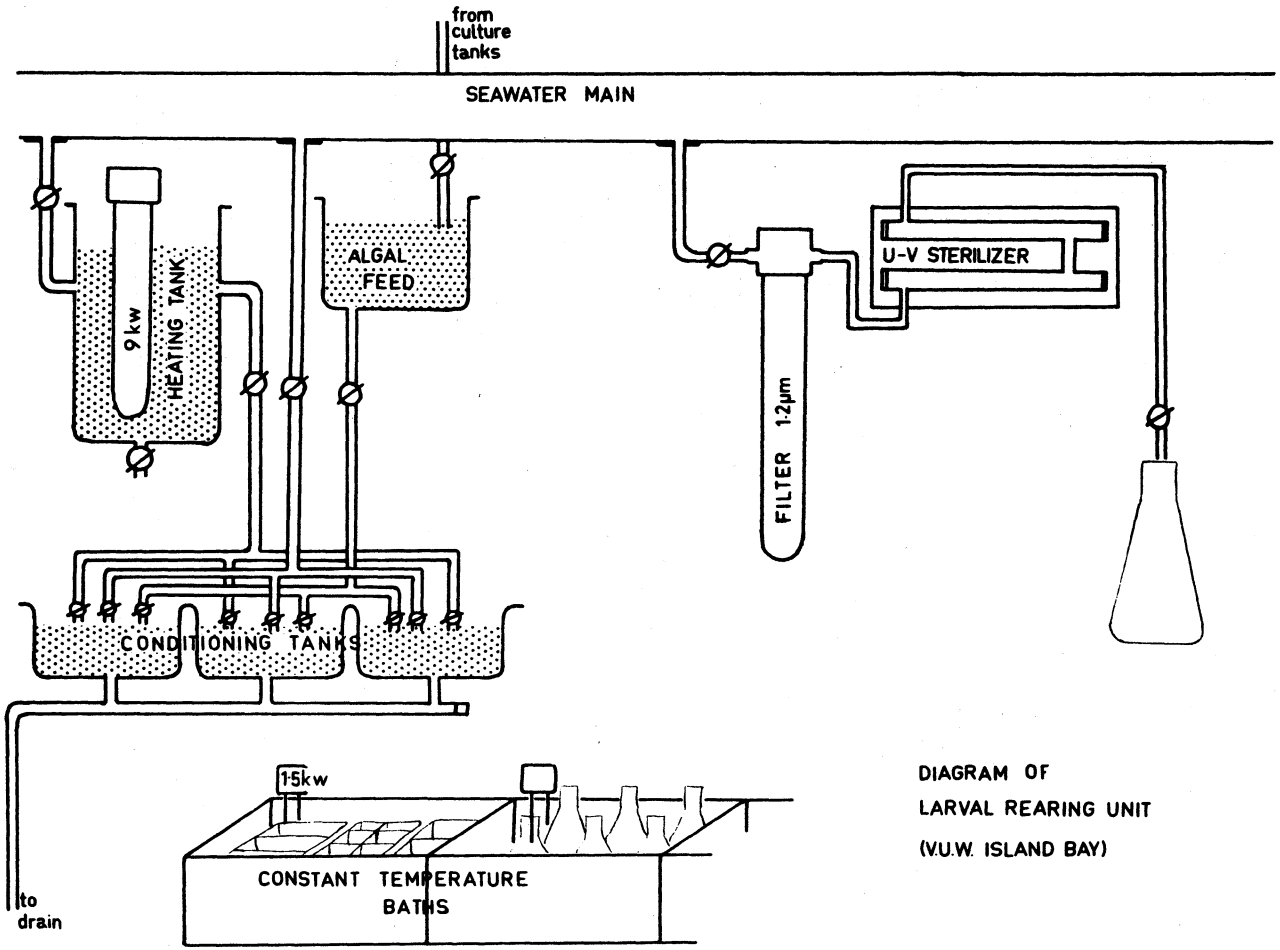


Fig. 1. Larval rearing unit consisting of a conditioning rack with heated and cold seawater, a supply of filtered and sterilized seawater, and constant temperature baths.

The constant temperature baths used for incubating the larvae were heated individually by 1.5 kW Inconel immersion heaters. Temperatures were maintained within ±2°C. Each bath was covered by a clear perspex dome which conserved heat while avoiding water loss through evaporation.

Water for use in larval cultures was first passed through a Millipore cartridge filter (Type Millitube PF/MF) of 1.2 µm pore size, and then sterilized by an ultra-violet unit as described by Loosanoff & Davis (1963). Water was pumped through the system at a maximum rate of 80 lh^{-1}, but slower with older filters. The stainless steel filter housing showed signs of some corrosion and leaking but no contamination was detected by bacteriological tests (incubation of sample on agar).

Axenic cultures of *Isochrysis galbana* and *Monochrysis lutheri* were grown from innocula supplied by the Fisheries Research Division of the Ministry of Agriculture and Fisheries. Glass carboys of 15 l capacity were used for the main cultures. Phillips TL65W/34RS fluorescent tubes continuously illuminated the cultures and also helped to maintain a temperature of about 19°C. Compressed air filtered through a cellulose-acetate filter (0.8 µm) was used for agitation. Provasoli (Provasoli *et al.* 1957), Oregon University (Malouf 1970) and Erdschreiber (Walne 1966) culture media have long preparation times but Vegemite Yeast Extract (Table 1) proved an excellent substitute. It supported good algal growth comparable to the more conventional media and was less demanding in preparation. A stock solution was obtained by dilution with equal parts of distilled water after gentle warming. This solution was used at a rate of 2.0 ml l^{-1} of culture medium. A chemical analysis of Vegemite appears in Table 1.

TABLE 1. APPROXIMATE ANALYSIS OF VEGEMITE, A CONCENTRATED AUTOLYSED EXTRACT MADE FROM *SACCAROMYCES CERVISIAE* AND OTHER BREWERS' YEASTS (AS SUPPLIED BY THE MANUFACTURERS - KRAFT FOODS PTY LIMITED).

Moisture	36.0%		
Nitrogen	4.5%		
Protein (N x 6.25)	28.6%	Sodium chloride	9.6%
Ash	15.9%	Phosphorus pentoxide	2.4%
Carbohydrates	9.9%	Potassium monoxide	3.3%
Reducing sugars	1.3%	Magnesium oxide	0.3%
		Calcium oxide	0.3%

Approximate Vitamin Content (per g)

Thiamine	140 µg	Choline	5000 µg
Riboflavin	142 µg	Biotin .05-.07	100 µg
Nicotinic acid	1000 µg	Inositol 1.2	10 µg
Pantothenic acid	50 µg	Pyrodokine	15-20 µg
Folic acid	20 µg		

Another algal culture suitable for adult shellfish only, was grown in household baths on the roof of the laboratory by adding commercial fertilizer to seawater, following Loosanoff & Engle (1942). There was no control over the organisms cultured, the amount of light or the temperature, and the cultures were poor in winter.

SPAWNING INDUCTION

Adult mussels (*Mytilus edulis aoteanus, Perna canaliculus* and *Aulacomya maoriana*) were collected from natural populations in the Marlborough Sounds during 1973-74 (see Tortell 1976a) and transported to the laboratory under damp and cool conditions. There the mussel shells were thoroughly cleaned by scraping, rinsing in freshwater and drying, before placing individuals in trays ready for spawning. Seawater in the spawning trays was filtered, sterilized and at ambient temperature (15-16°C). Also, it contained $0.05 g l^{-1}$ streptomycin sulphate and $0.03 g l^{-1}$ penicillin.

Mussels which subsequently failed to spawn were stimulated by other methods described in the literature. Groups of ten cleaned mussels were subjected to one of the following treatments:

1. Placed undisturbed in treated water at ambient temperature and light conditions (control).
2. Placed in treated water at 24°C (Loosanoff & Davis 1950).
3. Shaken vigorously in a bucket, then as in (2) (Field 1922, Young 1945).
4. Injected with KCl (Bayne 1965, Iwata 1950), NH_4OH (Sagara 1958).
5. Adductor muscle pricked, then in treated water at ambient temperature (Loosanoff & Davis 1963).
6. Adductor muscle torn, then in treated water at ambient temperature (Loosanoff & Davis 1963).
7. Valves wedged open (Loosanoff & Davis 1963).
8. Electric shock applied (Iwata 1950, Sugiura 1962).
9. Left dry for 8 hours, then in treated water at ambient temperature.
10. Left under continuous bright light in treated water at ambient temperature.
11. Incubated with the addition of sperm suspension.

Mussels not ready to spawn were placed in the conditioning tanks, the temperature raised from ambient to 22°C at the rate of 1°C day^{-1}, and maintained at this temperature for two weeks. During this time they were fed regularly with the crude algal culture. This method has successfully conditioned other bivalves (Davis & Chanley 1955, Loosanoff & Davis 1950, 1952, Malouf 1970, Walne 1964a).

CULTURE TECHNIQUES

Spawned gametes from individual mussels were collected separately. Sperm suspension was added to a 500 ml flask containing ova in sterile, antibiotic-treated water. After mixing and allowing the ova to settle back on the bottom of the flask, the water and excess spermatozoa were decanted off. Clean water was then added and the process repeated until most material in suspension was removed. The developing zygotes were then left undisturbed (apart from regular sampling) in culture flasks maintained at about 17°C for 48 h. Larval densities at this stage were about 2000 ml^{-1}.

After 48 h large particles were removed by pouring the culture through a 120 µm nylon sieve and the larvae collected on a 60 µm sieve. The concentrated larvae were then rinsed with sterile treated water and returned to a clean flask containing arbitrary amounts of *I. galbana* and *M. lutheri* cultures. The flagellate densities were not determined. Every 48 h thereafter the larvae were collected on a nylon sieve of appropriate size and rinsed with treated, and sterilized seawater. They were then returned to sterile flasks with treated and sterilized seawater containing food algae, and maintained at about 17°C in the constant temperature baths.

RESULTS

MYTILUS EDULIS AOTEANUS

There was no difficulty in obtaining gametes from *M. edulis*, in March, August and September 1973; they often spawned spontaneously while in transit to the laboratory. Adventitious spawning was usually prevented by keeping mussels out of water in a cool damp place with minimum handling. Spawning normally started within 20 min of reimmersing in water and 100% spawning was achieved after 4-5 h. It usually occurred at night or in early morning. Ova measured approximately 65 µm in diameter and were emitted in short orange rods, breaking up and settling quickly on the bottom. Spermatozoa (Fig. 2a) were ejected in dense milky white clouds and remained in suspension. The length of the sperm head (including acrosome) was approximately 7 µm and the tail was 65 µm long. Four batches of *M. edulis* larvae were reared to differing stages, the September batch being the most successful and surviving longest.

The first polar body (Fig 2b) was extruded after fertilization and the polar lobe (Fig. 2c) was fully developed

after 1.5 h. Extrusion of the second polar body was not observed. The 2-cell stage (Fig. 2d) was reached by 2.5 h and after a further 30 min the embryo consisted of 4 cells (3 micromeres and a single large macromere) (Fig. 2e). At 4 h the 8-cell stage (Fig. 2f) passed and the blastula (Fig. 2g) formed after 6 h. Ciliated gastrulae (Fig. 2h) showing slight rotation were seen 8 h, and the trochophore stage (Fig. 2i) 20 h after fertilization. The stomodaeum appeared soon after and subsequently the shell gland. Development proceeded to the veliger stage (Fig. 2j) by 40 h when the greatest length measured 120 µm. The straight-hinge stage occurred after 20 days (Fig. 2k) when an early umbo was discernible. This was distinct at 30 days when the larvae measured approximately 280 µm. Eyespots appeared after 45 days and nylon monofilaments were provided as a settlement surface. After 90 days the larvae (Fig. 2l) had neither settled nor metamorphosed and the experiment was terminated.

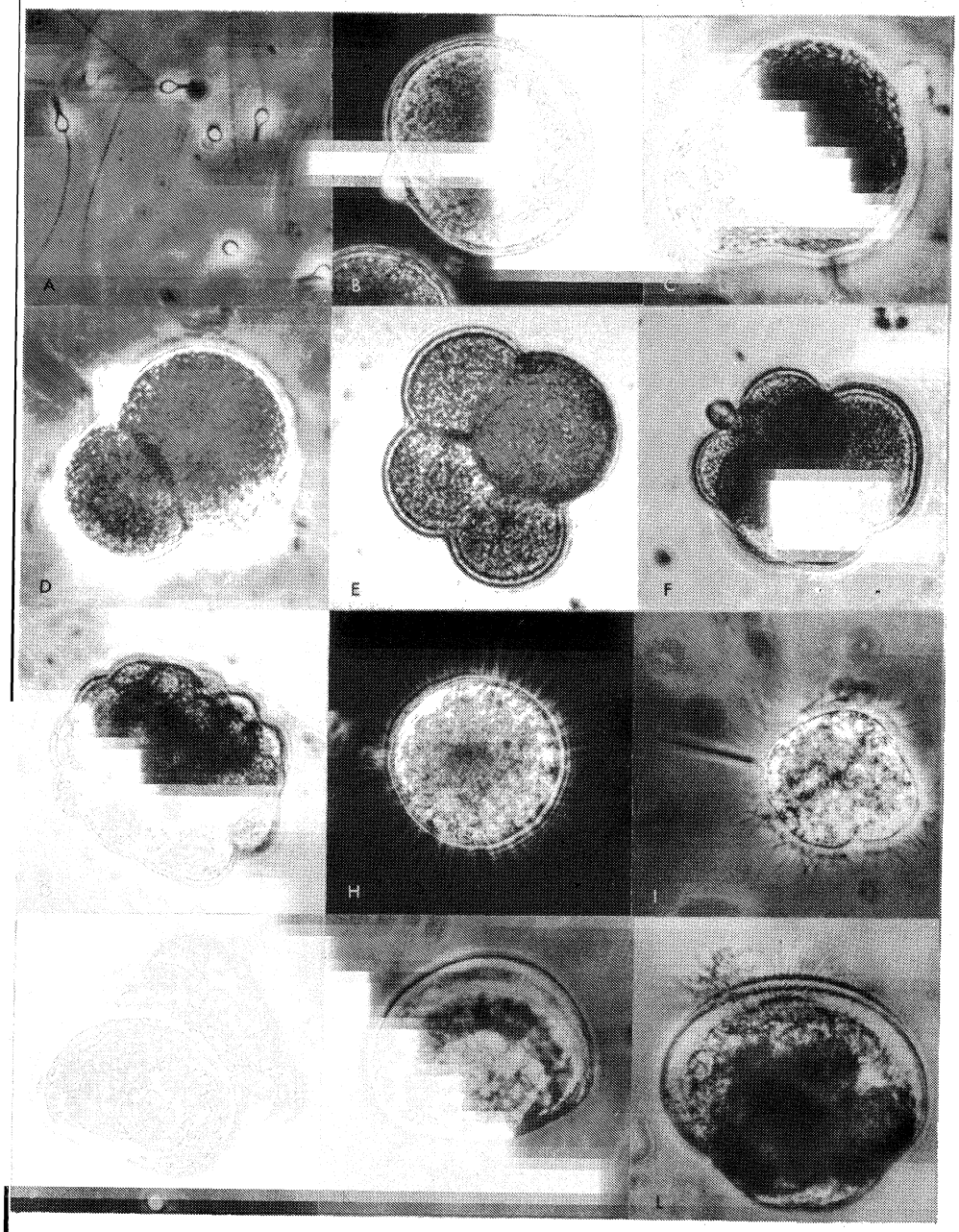
AULACOMYA MAORIANA

A. maoriana did not spawn after cleaning, nor after stimulation by placing in water at 24°C and so they were returned to running seawater at 16°C. After 15 h it was thought that some gametes had been released and after rinsing, the mussels were placed in spawning dishes where all spawned overnight. Spawning was not repeated with *A. maoriana* and only one batch of larvae was reared.

The eggs (Fig. 3b) were fertilised but the early stages of development were not observed. After 13 h most had reached the ciliated gastrula stage (Fig. 3c) and were very mobile. An excess of sperm was still present in the culture and a number of morphologically abnormal larvae were present, perhaps due to polyspermy. All but the free-swimming larvae were discarded and the remainder reached the trochophore stage (Fig. 3d) within 33 h and the veliger stage (Fig. 3e) within 60 h. On the fourth day a dark glandular area was obvious in the central region of the animals (especially Fig. 3f). This was not seen in the larvae of *M. edulis* although they had been fed on the same flagellate cultures. After 8 days most of the larvae lost the straight-hinge but there was a great variation in size. The prodissoconch stage (Fig. 3h) was reached on the 15th day and by the 24th day some individuals were up to 300 µm in length. There was still no sign of foot development and the majority had succumbed to ciliate infection. The experiment was terminated after 30 days without settlement or metamorphosis taking place, even though nylon monofilaments were present.

PERNA CANALICULUS

P. canaliculus failed to respond to any spawning stimulation even after conditioning. The fact that spawning took place on two separate occasions prior to cleaning and stimulation indicates that the mussels were ripe. Scraping the shell clean and placing in sterile treated water was carried out for all the induced groups and either or both of these procedures could have inhibited spawning.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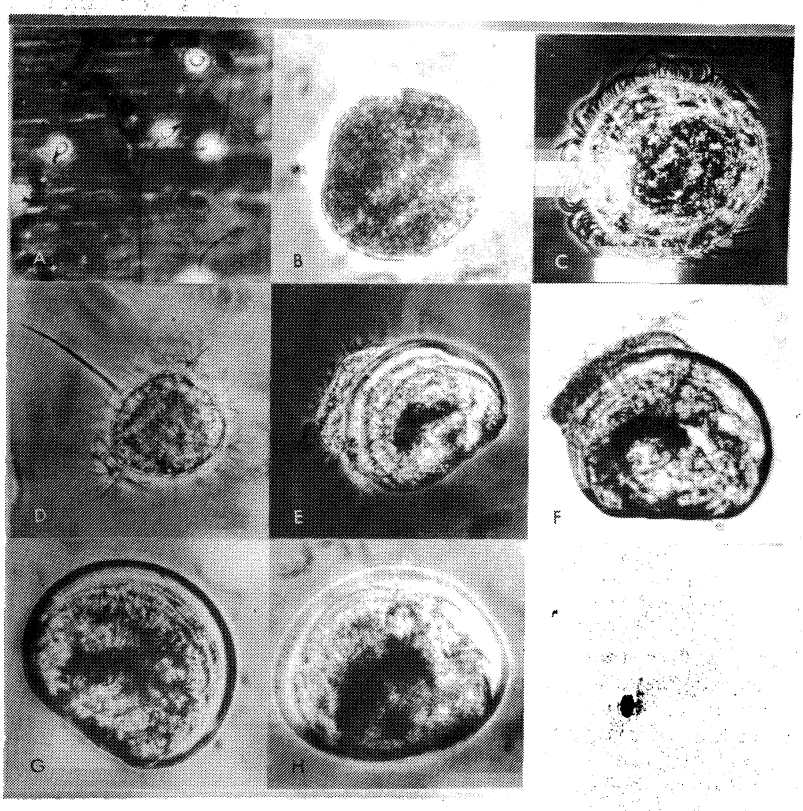

Fig. 3. Larval development of *Aulacomya maoriana*

a) Spermatozoa (total length c. 58 µm).
b) Ovum (c. 55 µm) with first polar body extruded.
c) Ciliated gastrula (13 h).
d) Trochophore larva (33 h).
e) Early veliger (60 h), c. 80 µm.
f) Later veliger (4 days) showing dark glandular area.
g) Late veliger (12 days), c. 200 µm.
h) Prodissoconch larva (24 days), c. 300 µm.

Fig. 2. Larval development of *Mytilus edulis aoteanus*

a) Spermatozoa (total length c. 73 µm).
b) Ovum (c. 65 µm) with first polar body extruded (1 h).
c) First polar lobe (1.5 h).
d) 2-cell stage (2.5 h).
e) 4-cell stage (3 h).
f) 8-cell stage (4 h).
g) Blastula (6 h)
h) Ciliated gastrula (8 h).
i) Trochophore larva (20 h).
j) Veliger larva - straight hinge (40 h, c. 120 µm).
k) Veliger larva - early umbo (20 days, c. 280 µm).
l) Late veliger larva (90 days, c. 320 µm).

Spontaneous spawnings in the laboratory were utilized but cultures raised from uninduced spawnings (hence not under strictly controlled conditions) were invariably contaminated and did not survive long. Ova were perfectly spherical and approximately 68 µm in diameter while spermatozoa had a total length of approximately 55 µm, of which 5 µm was the head. The 2-cell stage was reached after 1.5 h and after 2.5 h some 8-celled embryos were observed. Some embryos reached the ciliated gastrula stage after 12 h but looked abnormal and misshapen. No flagellated trochophores were seen after 60 h when the culture was discarded.

DISCUSSION

"Rearing larvae is often the most difficult part of marine cultivation" (Mason 1972). Experimental conditions which are apparently identical often produce different results (Walne 1956). Failure to rear bivalve larvae in the laboratory could be due to poor handling facilities for eggs and larvae, lack of proper food, mortality caused by parasites or pathogens (Loosanoff & Davis 1963), or lack of a suitable settlement substrate (Bayne 1965).

Reasons for the failure to rear larvae of any of these three species to or beyond the settlement stage are not known. All gametes used in these experiments were shed normally by the parents since stripped gametes often lead to abnormal embryos (Loosanoff & Davis 1963, Walne 1964a). Although no control was exercised over the quality of water (apart from sterilization and treatment with antibiotics) or the ambient environment, this should not have hindered larval development. Oxygen content and inorganic ions of the water as well as light intensity, seem to have no effect on delay in metamorphosis (Bayne 1965). Temperatures should not have influenced development (17°C ± 2°C) (Loosanoff & Davis 1963). The method used for changing the water and washing the larvae seemed less traumatic than that of Loosanoff and Davis (1963). Davis (1953) and Loosanoff & Davis (1963) showed that larval densities above 1000 ml^{-1} may impede development. Cultures in this study often started with densities of about 2000 ova ml^{-1}, but up to 50% of embryos were discarded at the first water change. Larval density could however, still have been too high.

White (1937) reared *M. edulis* larvae on *Nitzchia* and Loosanoff *et al.* (1954) state that they will thrive on most micro-organisms provided they are small enough to be ingested. *Isochrysis galbana* and *Monochrysis lutheri* were used in the present experiments. These two algae are not only small enough, but they also have no cell wall and produce little, if any, toxic external metabolites (Davis 1953, Davis & Guillard 1958, Walne 1970). However, an excess of algal food could well hinder development and this was not checked during the present experiments.

As a rule gametes were obtained from parents that had been cleaned and placed in sterile water treated with antibiotics

before spawning commenced. Resulting embryos appeared healthy and active, and no parasites or pathogens were noticed in the cultures. Whenever gametes were taken from holding tanks containing uncleaned mussels, contamination with ciliates was evident, and often the embryos were abnormal and soon died. Parasites and pathogens were eliminated by preparing the parents and by avoiding gametes spawned under uncontrolled conditions.

The substrates available for settlement in the present experiment were monofilament nylon threads and the glass sides of the container. Bayne (1965) found that mussel larvae were capable of attachment a considerable time before they will settle on glass and that the majority die before doing so. He also found that filamentous algae were a successful substrate while nylon filaments were extremely unsuitable. Since mussel larvae settled well on polypropylene filaments in the field during the present experiments (see Tortell 1976a, b), the same material was offered to the larvae in the laboratory, but without success. Synthetic filaments in the field have a chance of being 'conditioned' by epiphytes while the laboratory filaments were sterilized before introduction among the larvae. This could well be the reason for their failure to attract any larvae.

The present experiment was partially successful in rearing the larvae of *M. edulis* and *A. maoriana* but these larvae failed to metamorphose. The most likely causes of failure were high larval densities, an excess of food organisms and the lack of suitable settlement substrates.

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